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ISOLATION AND IDENTIFICATION OF SOME ENTEROBACTERIAL SPECIES FROM CHILDREN WITH DIARRHOEA WHOM ATTENDING THE AL-GOURDA HEALTH CENTRE IN SABHA CITY AND STUDYING THEIR ABILITY TO PRODUCE PROTEASE ENZYME

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ABSTRACT

This study included the isolation and identification of some bacterial species causing diarrhea in children under five years old. Ninety eight stool samples were collected from children attending the Al-Gourda Health Center in Sabha for the period between February 2022 to September 2022. Samples were cultured on selected media and the growing colonies were identified using morphological characteristics and biochemical tests. fifty eight bacterial isolates belongs to the family enterobacteriaceae were identified, 46.6 %(27 isolates) of them were *E.coli*; 20.7% (12 isolates) were *Salmonella*; 15.5 %(9 isolates) of *Shigella*; 10.3% (6 isolates) of *Klebsiella;* 5.1% (3 isolates) and 1.7% (one isolates) of *Pseudomonas.* Protease production, which is considered as one of the factors enhancing inflammatory bowel disease, was investigated and it has been found that all the isolates used in the study were able to produce the enzyme.

KEYWORDS: Bacteria, Children, Diarrhea, Al-Gourda Health Center.

INTRODACTION

The most prevalent type of gastrointestinal infection is diarrhea, which is characterized as an increase in the frequency of bowel movements and the rapid development of regular, more or less fluid intestinal evacuations. The proximal small intestine, the part of the colon where more than 90% of physiologic net fluid absorption takes place, was discovered to be the site of diarrhea's production by pathogenic mechanisms.[1,2] Gastrointestinal disorders are regarded as prevalent illnesses worldwide, the second most frequent reason for doctor visits, and the leading cause of baby and young child mortality in the developing world. In Latin America, Africa and Asia, it has been estimated that a child's likelihood of dying from diarrheal sickness before the age of five can be as high as 50%, depending on nutritional and socioeconomic conditions. Diarrhea is a factor in one-fourth of baby and toddler deaths in underdeveloped nations.[2,3,4,5] Many different types of microorganisms can cause diarrheal illnesses, which can occasionally result in dysentery, a kind of diarrhea in which the stools are bloody, and mucus filled. It was once believed that only Salmonellae and Shigellae produced diarrheal illnesses, however it is now known that several strains of Escherichia coli also cause these symptoms. In addition Campylobacter, Vibro, Aeromonas, and Plesiomonas, diarrheal illnesses have been linked to these microbes.[6] Wadgaonkar et al. (2005) reported on the effect of breastfeeding on the severity of illness in diarrheal diseases and suggested that it significantly modifies the spectrum of severity in diarrheal diseases from severe to non-severe illness.[7,8] A significant number of gram-negative bacilli belonging to the intestinal family, many of which are the primary causes of infections in the human gastrointestinal system that result in diarrhea are Shigella, Salmonella, and Escherichia. One of the most typical factors that causes gastroenteritis, The production of endotoxins, which are represented by the layer of polysaccharides, fatty acids and enterotoxins linked to cases of diarrhea, as well as the production of numerous types of the capsular layer that allow it to inhibit the process of phagocytosis, are all examples of the virulence of bacterial species belonging to the family Enterococcus.[9,10] Enterobacteria, which can withstand the killing effects of digestive enzymes and pH changes, cause the infection when consumed in food and beverages.[11,12] The pathogen enters the intestine through the mucous layer and begins to multiply. Numerous pathogens have the ability to create enterotoxins that result in diarrhea and subsequent infections, particularly in cases of intestinal transit.[13]

The protease enzyme's function as a virulence factor

Proteases are enzymes that break down proteins and peptides. They are found in all kingdoms of life. Typically, these enzymes either hydrolyse the peptide bonds inside of proteins or cleave the bonds close to the amino- or carboxyl-terminal ends of proteins. Although occasionally the infected host is poisoned by the infectious species' enzymes.[14,15] A bacterial pathogen's normal infection cycle has the following stages: selecting and invading a suitable host; dispersing inside the host organism; the emergence of disease signs; and lastly the transfer to a new host. Each stage must be successfully completed with the help of several factors that the pathogen produces and refers to as virulence factors. These molecules are directly in charge of the host's colonization, escape from the host's defences and development of illness symptoms. They specifically help the pathogen adhere to the host surfaces, dismantle physical barriers like cell walls or intercellular junctions, mediate pathogen migration, modify the host's immune response and many other things. Each stage of the pathogen's life cycle involves exposure to a range of potentially stressful biotic and abiotic conditions.[16] Proteases plays various important roles in the mechanisms of virulence over the whole infection cycle of the pathogen. Secreted proteases facilitate penetration and efficient dissemination within the host by participation in the degradation of the host's physical barriers.[17] Proteolytic enzymes also undermine the host's defenses, facilitating colonization of the host. Numerous proteases have regulatory roles that enable the pathogen to respond appropriately to environmental changes and to initiate infection when the bacteria need it most. Proteases are crucial parts of the system that regulates protein quality and keeps cellular proteostasis in check. Protein misfolding and denaturation are just two effects that occur when a cell is exposed to stressful situations. In order to prevent the proteins from aggregating or malfunctioning, the irreversibly damaged proteins are eliminated from the cell by proteolysis.[18,19]

MATERIALS AND METHODS

The study was conducted on children under five years of age who suffer from diarrhea and stomach pain, whom attending the Health Centre in Al-Gourda, Sabha between February 2022 to September 2022.

Sample Collection

A total of 98 stool samples were collected from children under five years of age attending the Health Centre in Al-Gourda, Sabha. The stool samples were collected by their mothers in sterile, transparent and wide mouthed bottles and transported to the microbiology laboratory in a cool box for immediate examination.

Processing of samples

Culture

A sterile loop was dipped into stool sample to obtain an inoculum which was streaked on to the surface of the plate containing sterile MacConkey agar, Eosin Methylene Blue Agar plates and Salmonella –shigella agar using standard method of Prescott and Harley, (2005).[20] The procedure was applied for each sample and the plates were incubated at 37°C for 24 hours. The presumptive colonies of each isolate on agar plates were further subcultured to get a pure culture. The covered pure isolates were preserved for further bacterial identification.

Characterization and identification of isolates

The bacterial isolates were identified based on colonial morphology, cultural characteristics and biochemical tests. Gram staining was done for each individual isolates according to the method described by Holt *et al*., (1994) and Sherman, (2005).[21,23] The isolates were also characterized by biochemical tests. i.e., oxidase test, urease test, citrate test, Indole test, Methyl red test, Catalase test, fermentation test, by standard methods given by Sherman, (2005) and Holt *et al*., (1994).[21,23] Based on culture results and microscopic and biochemical characteristics, bacterial isolates were identified to the genus level.

Identification of proteolytic bacteria

The activity of proteolytic bacteria was tested qualitatively on skimmed milk media, indications that microbes can integrate protein (casein) which are shown through clear zones around the colonies. Petri dishes containing skimmed milk agar were inoculated with bacterial isolates with three replicates for each bacterial isolate. The inoculation was done in the centre of the dish and in a circular shape with a diameter of 1 cm, then the dishes were incubated at a temperature of 37°C for 24-48 hours. The appearance of a transparent area around the bacterial growth is an indicator of the breakdown of casein by the action of protease enzyme.[24]

RESULT

Isolation and diagnosis

The Cultural characteristics of the isolates on agar plate is presented. The result showed how the isolate were characterized on the basis of colony morphology and staining characteristics. It was observed that all the isolates were Gram negative i.e. pink coloured and morphologically are small rod in shape which are arranged in single or paired under the microscopic examination. The Biochemical characterization of the isolates showed how isolates were characterized on the basis of biochemical identification. Biochemical test include Indole, Methyl-red, Vogues-Proskauer, Citrate utilization test, Lactose fermentation, Oxidase and Catalase test. The number and percentage of isolates from stool samples is presented in Table 1. A total of fifty eight 58(59.2%) isolates were recovered from ninety eight (98) stool samples. The results obtained from the data shows that the bacteria found in the stool samples were *Escherichia coli 27 (46.6%)*, followed by *Salmonella species 12* (20.7%), *Shigella species 9* (15.5%), *Klebsiella specie 6* (10.3%), *Proteus species 3* (5.1%), and *Pseudomonas spp 1*(1.7%).

Table 1: Percentage occurrence of the bacteria isolates in faecal samples.

The Isolate	No. (%)
Escherichia coli	27 (46.6%)
Salmonella spp	12 (20.7%)
Shigella spp	9 (15.5%)
Klebsiella spp	6 (10.3%)
Proteus spp	3 (5.1%)
Pseudomonas spp	1(1.7%)
Total	58 (100%)

The ability of bacterial isolates to produce protease

Eight isolates bacteria (*E. coli* 3, *E. coli* 12, *E. coli* 23, *Salmonella spp 4, Shigella spp 2, Pseudomonas spp* 1 , *Klebsiella spp 1, Proteus spp 2*) were selected randomly to make a comparison for production of protease enzyme. All the isolates used in this study were protease producer when they had been grown on skim milk agar and incubated at 37°C for 24-48 hrs. The proteolytic zone around the bacterial growth was very clear and could be simply detected. The diameters of zone were varied from one isolates to another, *E. coli* 3 was given a very large zone (2.8 cm); while the others were about (0.5- 2.3 cm) as shown in Table (3).

Table 3: The ability of bacterial isolates to produce protease enzyme on skim milk agar at a temperature of 37°C for 24-48 hours.

The Isolate No	Decomposition zone diameter (cm)*
E. coli 3	2.8
E. coli 1	2.3
E. coli 2	2.1
Salmonella spp 4	1.4
Shigella spp 2	1.2
Pseudomonas	1.2
Klebsiella spp 1	0.8 cm
Proteus spp2	0.5 cm
* The readings are the average of three replicates	

DISCUSSION

Enteric bacteria are microbes that reside in the guts of animals and humans. However, there are some among them that reside in intestinal tracts of animals which can then cause diseases and harsh reactions when humans become infected with them.[25] They can cause a mild infection, such as food poisoning or severe community-infections like diarrhea. Such examples of enteric bacteria include *Salmonella, Escherichia coli, Shigella, Klebsiella, Campylobacter, Enterobacter Yersinia, Vibrio and Citrobacter*.[26] Few studies have been conducted to determine the etiology of childhood diarrhea in Libya.[27,28,29] In this study fifty eight (58) isolates (6 genera) were isolated from 98 stool samples and they were characterized on the basis of biochemical identification. The result obtained from the data shows that the bacteria found in the stool samples were *Escherichia coli, Salmonella spp, Shigella spp, Klebsiella spp, Proteus spp and Pseudomonas spp* (Table 1). Between the 58 pathogenic bacteria isolates, *27* (46.6%) isolates are related to *Escherichia coli*. Our findings were in line with other reports in Libya and other countries indicating that bacterial pathogens are important contributors to ediatric diarrhea, and enteropathogenic strain of *Escherichia coli* is the most frequently detected pathogen.[1, 3, 7, 13, and 15] *E.coli* is a normal inhabitants of the colon and therefore, determination of the clinical significance of this isolation depends on the determination of whether the isolated strains were pathogenic or not. Identification of pathogenic *E.coli* needs either serotyping which is easy, cheap and available or more sophisticated techniques like molecular methods. However, due to lack of our resources in this study, no determination of pathogenicity of *Esch.coli* was made. Moreover, viral studies are needed to exclude a viral cause for diarrhea in these with gastroenteritis. *Salmonella* isolates from cases of diarrhea were found in 12 (20.7%) of the patients and *Shigella* isolates in 9 (15.5%), which is a higher rate than in earlier investigations conducted in Libya.[30] Environmental pollution and use of polluted water are responsible for infecting kids, especially babies. These factors may also play a significant part in the spread of this disease among kids who rely on artificial feeding, since these kids are more vulnerable to infection.[31] The study also noted a 6 (10.3%) species isolation rate for *Klebsiella*. The bacteria normally exists at a rate of (5–35.5%) in the gastrointestinal tract, and its endemic incidence rises in hospitalized patients, especially those who are immunosuppressed. Rassol and his team's 2003[38] investigation revealed the involvement of this bacteria in the spread of epidemic diarrhea due to a variety of One of its strains has the capacity to acquire plasmids from E. coli, and a study conducted in 2001[32] by Braun and his team revealed the contribution of this germ to the development of intestinal inflammation. Numerous virulence elements in this germ improve its pathogenicity and enable it to enter human tissues.[33] The positive result of the protease enzyme test showed the bacterial isolates have a clear ability to hydrolyze milk protein (casein), and this is consistent with previous studies, for example, the study of Matsumoto et al. (1984)[34], showed the ability of Proteus mirabilis species and the genus Salmonella to produce protease enzyme. The ability of the isolated bacteria to produce protease enzymes increases their virulence and ability to invade host tissues. As these enzymes destroy immunoglobulin IgA, which is the first line of defense for the epithelial layer lining the intestinal tract and respiratory tract, as well as destroying immunoglobulin IgG and the proteins of cell membranes. It was found that the proteolytic enzymes of the bacterium Proteus mirabilis have the ability to dissolve the membranes of the intestinal cells of mice, which leads to the exit of fluids and salts into the intestinal cavity with the occurrence of bleeding, which results in the occurrence of intestinal diarrhea.[35, 36] The study of Cenac and his group (2002)[37] showed the presence of receptors for protease enzymes. On the surface of the intestinal epithelial cells widely, and the

study indicated that stimulating these Receptors using trypsin enzymes play a role in causing inflammatory bowel disease of the colon. It was shown that there were clear variations in the efficacies of the production of protease from different species and different sources, these variants were due to differences of genetic ability among isolates.[38]

CONCLUSION AND RECOMMENDATION

Stool samples from children under the age of five attending the Health Centre in Al-Gourda, Sabha, Libya were examined for enteric bacteria. *Escherichia coli*, *Salmonella spp*., *Shigella spp.*, *Klebsiella spp.*, *Proteus spp.*, and *Pseudomonas spp*. isolates were identified by cultural characteristics, Gram staining, and biochemical testing. Escherichia coli was the most typical strain found, according to this investigation. The results show that the infection is connected to the intestinal bacteria. We advised that health education is crucial to raise knowledge about the connection between unsanitary food handling and preparation and food born illnesses. The study showed the ability of most of the isolated strains to secrete the protease enzyme, which indicates the role of this enzyme causes an inflammatory bowel condition.

REFERENCES

1. Ryan K.J, and Ray C.G: Sherris Medical Microbiology An Introduction to Infectious Diseases: Enteric Infections and Food Poisoning. 4th (Ed.). McGraw Hill, 2004; 609, 857, 860–861.
2. Chamberlain N.R: Medical Microbiology The Big Picture: Gastrointestinal Tract and Liver. McGraw Hill Lange, 2009; 169–237.
3. Gillespie S, and Bamford K: Medical Microbiology and Infection at a Glance: Bacterial Diarrhoeal Disease. Blackwell Sciences Ltd, 2000; 98–99.
4. Bruyn G.D, and Bouckenooghe A: Evidence – Based Infectious Diseases: Diarrhea. 2nd (Ed.). Blackwell Publishing Ltd, 2009; 98–114.
5. Manning S.D: Deadly Diseases and Epidemics: Escherichia coli Infections. 2nd (ed.). Chelsea House Publishers, 2010; 29.
6. Tesh, V. and O Brien, A. O. The pathogenic mechanisms of Shiga toxin and the Shiga – like toxins. Molecular Microbiology, 1999; 5: 1817–1822.
7. Wadgaonker, S.P., Baholikar, A. V., Wadia, R.S., Sharma, V. V. and Shukla, R.N. Shigellosis in Poona. Journal Associates physician, 2005; 25: 13–19.
8. Jepson, J.H., per- cee, J.H., Rooker, D.W. and Turner, J.S. (1980). Shigellosis in Graeme S.A. (1978). Drug treatment: Principle and Practices of Clinical. Pharmacological Theraputics. London, 5: 123-126.
9. Talaro, K. and Talaro, A. (1996). Foundation in Microbiology. 2nd ed. W.M.C. Brown publisher USA, 394.
10. Nester, E. W.; Anderson, D. G.; Robert Jr., C. E. ; Pearsall, N. N. and Nester, M.T. (2004). Microbiology: A human prespective. 4th ed. McGraw – Hill Companies, Inc. Boston, 610.
11. Gillespie S, and Bamford K: Medical Microbiology and Infection at a Glance: Bacterial Diarrhoeal Disease. Blackwell Sciences Ltd, 2000; 98–99.
12. Southwick F: Infectious Diseases A Clinical Short Course: Gastrointestinal and Hepatobiliary Infections. 2nd (Ed.). McGraw – Hill Lange, 2007; 190–209.
13. Tortora, G.J.; Funke, R. B. and Case, C.L. (2004). Microbiology An Introduction; 8th ed. Person Education, Inc.
14. Harrington, D. J. Bacterial collagenases and collagen-degrading enzymes and their role in human disease. Infect. Immun, 1996; 64: 1885–1891.
15. Thibodeaux BA, Caballero AR, Marquart ME, et al. Corneal virulence of Pseudomonas aeruginosa elastase B and alkaline protease produced by Pseudomonas putida. Curr Eye Res, 2007; 32(4): 373–386.
16. Vorholt J.A. Microbial life in the phyllosphere. Nat. Rev. Microbiol, 2012; 10: 828–840. doi: 10.1038/nrmicro2910.
17. Hueck C.J. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol. Mol. Biol. Rev, 1998; 62: 379–433.
18. Gasser B., Saloheimo M., Rinas U., Dragosits M., Rodríguez-Carmona E., Baumann K., Giuliani M., Parrilli E., Branduardi P., Lang C., et al. Protein folding and conformational stress in microbial cells producing recombinant proteins: A host comparative overview. Microb. Cell Fact, 2008; 7: 11. doi: 10.1186/1475-2859-7-11.
19. Gur E., Biran D., Ron E.Z. Regulated proteolysis in Gram-negative bacteria—How and when? Nat. Rev. Microbiol., 2011; 9: 839–848. doi: 10.1038/nrmicro2669.
20. Presscot, L.M, Harley, J.P. and Klein, D.A. (2005).Microbiology Sixth edition. McGraw Hill International edition New York.
21. Holt, J.G., Krieg, N.R., Senath, P.H.A., Staley, J.T. and Williams, S.T. (1994): Bergey's Manual of Determinative Bacteriology 9th Ed. Baltimore Md Williams and Wilkins.
22. Sherman, N.; Microbiology: A laboratory manual. Sixth Edition, ISBN, 2005; 81(3): 265-267.
23. Sharma, J.; Singh, A.; Kumar, R. & Mittal, A. Partial purification of an alkaline protease from Aspergillus oryzae AWT20 and its enhanced stabilization in entrapped ca-alginate beads. Int. J. Microbiol, 2006; 2(2): 98-106.
24. Singh, V., Pandey, P. C. and Jain, D. K. (2013).A text book of botany, fourth edition. Rastogi publication, India.
25. Kim, N., Juang, S. and Na, H. Enteric bacteria isolated from diarrheal patients in Korea in 2004.

Osong public health Res. Perspect, 2014; 6(4): 233-240.
26. Ali MB, Ghenghesh KS, Ben Aissa R, Abuhelfaia A, Dufani MA. Etiology of childhood diarrhea in Zliten-Libya. Saudi Med J, 2005; 26: 1759–1765.
27. Ghenghesh KS, Abeid SS, Bara F, Bukris B. Etiology of childhood diarrhea in Tripoli-Libya. Jamahiriya Med J, 2001; 1: 23–29.
28. Amal Rahouma, John D. Klena, Zaineb Krema, Abdalwahed A. Abobker, Khalid Treesh, Ezzedin Franka, Omar Abusnena, Hind I. Shaheen, Hanan El Mohammady, Abdulhafid Abudher, and Khalifa Sifaw Ghenghesh: Enteric Pathogens Associated with Childhood Diarrhea in Tripoli-Libya. Am J Trop Med Hyg, 2011 Jun 1; 84(6): 886–891.
29. Ghenghesh KS, Abeid SS, Bara F, Bukris B. Etiology of childhood diarrhea in Tripoli-Libya. Jamahiriya Med J, 2001; 1: 23–29.
30. Schultz, G.E.; Kirby, R.S.; Flick, E.L.; Stefanova, R.; Eisenach, K.D and Cave, M.D. Epidemiology and Molecular identification of Salmonella infection in children. Arch. Pediatr. Adolesc. Med, 1998; 152: 659-664.
31. Braunwald, E.; Hauser, S.L.; Fauci, A.S.; Lonho, D.L.; Kasper, D.L and Jameson, J.L. (2001). Harison's principles of Internal Medicine 15th ed. McGraw-Hill Company. USA.
32. Brooks, G.F.; Butel, G.S. and Morse, S.A. Jawetz, Melnick, & Adelberg's Medical Microbiology.23th ed. Lange Medical books/McGraw-Hill. New York, 2004; 28: 248-268.
33. Matsumoto, K.; Maeda, H.; Takata, K.; Kamata, R. and Okamura, R (1984). Purification and characterization of four proteases from a clinical isolate of Serratia marcescens Kums 3958. J. Bacteriol, 225-232.
34. Loomes, L.M.; Senior, B.W.; and Ker, M.A. A proteolytic enzyme secreted by Proteus mirabilis degrades immunoglobulins of IgA1, IgA2 and IgG isotypes. Infect. Immune, 1990; 58(6): 1979-1985.
35. Hume, E. B.; Conerly, L. L.; Moreau, J.M.; Cannon, B.M.; Engel, L.S.; Stroman, D.W.; Hill, J. M.; O'Callaghan, R.J. Serratia marcescens Keratitis: strainspecific corneal pathogenesis in rabbits. Curr. Eye. Res, 1999; 19: 525-532.
36. Cenac, N.; Coelho, A.; Nguyen, C.; Compton, S. ; Andrade-Gordon , P.; MacNaughton, W.K.; Wallce, J.L.; Hollenberg, M.D.; Bunnett, N .W. ; Gracia-Villeur, R. ; Bueno, L. and Vergnolle, N. Induction of intestinal inflammation in Mouse by activation of Proteinase-Activated reseptor-2. American J. of pathology., 2002; 161: 1903- 1915.
37. Al-Rubaei, B.L. (2001). Enzymatic study on the Protease produced by Proteus mirabilis causes urinary tract infections. M.Sc. Thesis. College of Science, University of Baghdad. (In Arabic).